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14. ABSTRACT
The BCR-ABL fusion gene, the hallmark of CML, plays a causal role in the development of CML. The BCR-ABL tyrosine kinase inhibitors have been successfully used to treat patients with CML, but residual disease persists and drug resistance emerges. Although BCR-ABL remains to be an attractive target for developing CML therapies, identifying and targeting additional essential components in the development of CML are important for overcoming resistance to BCR-ABL tyrosine kinase inhibitors and for eradicating leukemic cells. Substantial evidence indicates that Ras and Ras related proteins are critical mediators of BCR-ABL in leukemogenesis. Ras-converting enzyme (Rce1) and isoprenylcysteine carboxyl methyltransferase (Icmt) are two unique enzymes for Ras modifications that are critical for their functions. Targeted inactivation of Rce1 or Icmt is, therefore, an attractive strategy for the treatment of CML. The goal of this project is to determine whether targeted inactivation of Rce1 or Icmt could block BCR-ABL leukemogenesis. In this study, we have found that Icmt plays a critical role in BCR-ABL leukemogenesis and that the role of Rce1 in BCR-ABL leukemogenesis is dosage dependent. We have also found that the mutation affecting the AAX peptide cleavage and methylation of RAS in cis significantly reduces RAS leukemogenesis, suggesting that the AAX peptide cleavage and methylation play an important role for RAS leukemogenesis.

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Chronic Myelogenous Leukemia (CML)

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Report

Introduction

The *BCR-ABL* fusion gene, the hallmark of CML, plays a causal role in the development of CML. The BCR-ABL tyrosine kinase inhibitors have been successfully used to treat patients with CML, but residual disease persists and drug resistance emerges. This clinical time bomb will have to be diffused in the not so distant future. Although BCR-ABL remains to be an attractive target for developing CML therapies, identifying and targeting additional essential components in the development of CML are important for overcoming resistance to BCR-ABL tyrosine kinase inhibitors and for eradicating leukemic cells. Substantial evidence indicates that RAS and RAS related proteins, which are commonly activated in human cancers, are critical mediators of BCR-ABL in leukemogenesis. RAS-converting enzyme (Rce1) and isoprenylcysteine carboxyl methyltransferase (Icmt) are two unique enzymes for RAS modifications that are critical for their functions. Targeted inactivation of Rce1 or Icmt is, therefore, an attractive strategy for the treatment of CML. The goal of this project is to determine whether targeted inactivation of Rce1 or Icmt could block BCR-ABL leukemogenesis. In the past funding period we have generated mice with conditional alleles of Rce1 or Icmt and used these mice to evaluate the importance of Rce1 and Icmt in BCR-ABL leukemogenesis. Our preliminary results show that Icmt plays an important role in the pathogenesis of CML.

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1. (Task 1) We have generated $Rce1^{flx/flx}$ or $Icmt^{flx/flx}$ mice, as well as $Rce1^{flx/+}$, $Icmt^{flx/+}$, $Rce1^{+/+}$, or $Icmt^{+/+}$ control mice harboring the Mx1-Cre transgene through breeding $Rce1^{flx/+}$/Mx1-Cre and $Icmt^{flx/+}$/Mx1-Cre mice.
2. (Task 6) We examined the leukemogenic potential of BCR-ABL with or without Rce1 or Icmt using the mouse bone marrow transduction and transplantation model for CML as proposed. Briefly Cre expression was induced in $Rce1^{flx/flx}$/Mx1-Cre, $Rce1^{flx/+}$/Mx1-Cre, $Icmt^{flx/flx}$/Mx1-Cre, $Icmt^{flx/+}$/Mx1-Cre, or Mx1-Cre control mice by intraperitonieal injections of polyinosinic-polycytidylic ribonucleic acid (pI-pC) for 4 times. These mice were then treated with 5-fluoruracil (5-FU) for 4 days to enrich and activate hematopoietic stem cells. Bone marrow (BM) cells from the above mice were isolated and transduced with *BCR-ABL* retroviruses. As shown in Figure 1, BCR/ABL leukemogenic potential was reduced in pI-pC-induced $Rce1^{flx/+}$/Mx1-Cre mice, compared to BCR/ABL transduced $Rce1^{+/+}$/Mx1-Cre mice. However, BCR/ABL transduced $Rce1^{flx/flx}$/Mx1-Cre mice developed disease much faster then the control mice with wt Rce1. These data suggest that the role of Rce1 in BCR/ABL leukemogenesis is complicated and dependent on the dosage. This may due to the fact that there are many substrates of Rce1, some of them may facilitate BCR-ABL leukemogenesis, while others may inhibit. We will repeat this experiment and confirm the finding.

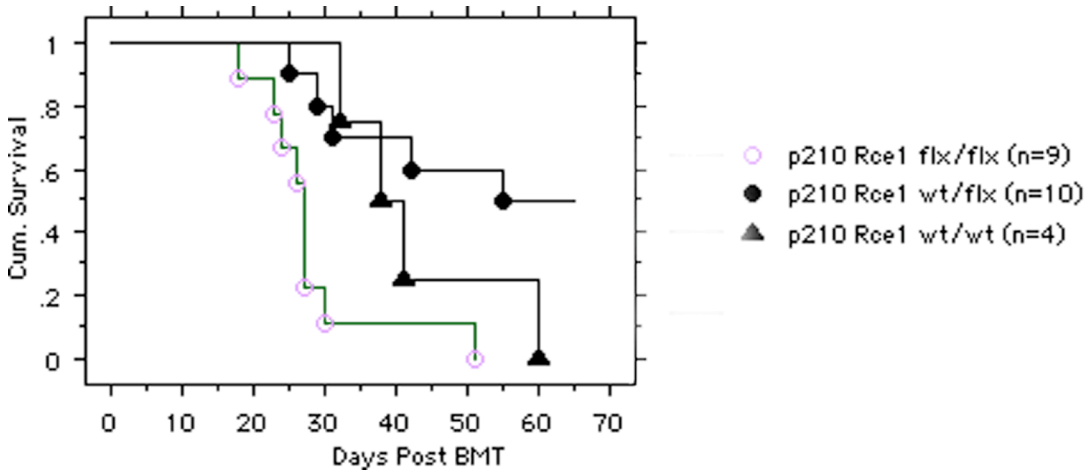

Figure 1. Survival of mice receiving transplantation of *BCR-ABL* transduced BM cells from pI-pC-induced $Rce1^{flx/flx}$/Mx1-Cre, $Rce1^{flx/+}$/Mx1-Cre, $Rce1^{+/+}$/Mx1-Cre mice. Survival curves were generated by Kaplan-Meier survival analysis. The number (n) of mice for each group is indicated.

Unlike complicated result of Rce1 inactivation, Icmt inactivation significantly mitigated BCR-ABL leukemogenesis (Figure 2). This result suggests that Icmt plays an critical role in BCR-ABL leukemogenesis and that Icmt may be an effective target for developing CML therapeutics.

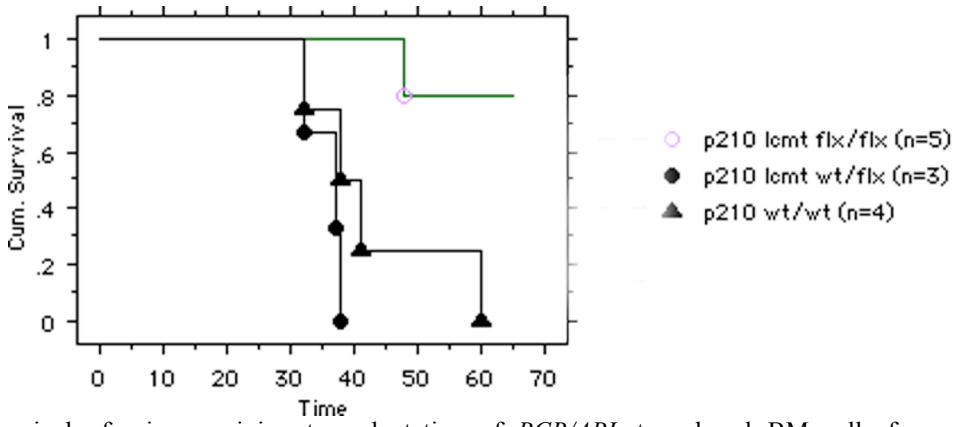

Figure 2. Survival of mice receiving transplantation of *BCR/ABL* transduced BM cells from pI-pC-induced $Icmt^{flx/flx}$/Mx1-Cre, $Icmt^{flx/+}$/Mx1-Cre, $Icmt^{+/+}$/Mx1-Cre mice. Survival curves were generated by Kaplan-Meier survival analysis. The unit of time is day post-BM transplantation. The number (n) of mice for each group is indicated.

3. (Related to Tasks 5 and 9). In addition to RAS proteins, a broad class of proteins, many of which are also involved in cellular regulatory processes that are important for tumor formation, are also substrates of Rce1 and Icmt. Rce1 and Icmt may affect BCR-ABL leukemogenesis through RAS proteins and/or other targets of the enzymes. To address whether Rce1 and Icmt affect BCR/ABL leukemogenesis through RAS, we have generated an A2 (the second alphatic residue in the CAAX motif) mutant of oncogenic RAS (NRASD12) for testing the importance of removal of the AAX tripeptide and methylation of the terminal farnesylated cysteine residue in RAS itself (this would avoid complications of targeting proteins other than RAS). The A2 residue has been shown to be important for the AAX peptide cleavage and methylation. Using the mouse bone marrow transduction and transplantation model system, we demonstrated that the A2 mutant of NRASD12 has significantly reduced leukemogenic potential- having a longer disease latency compared to NRASD12 mice ($P<0.0001$) (Figure 3). In addition, unlike NRASD12, which induces acute myelogenous leukemia (AML) in most recipient mice, the A2 mutant of NRASD12 only induces chronic myelomonocytic leukemia (CMML), a milder form of hematological malignancy (data not shown). The results indicate that the AAX peptide cleavage and methylation play an important role for RAS leukemogenesis.

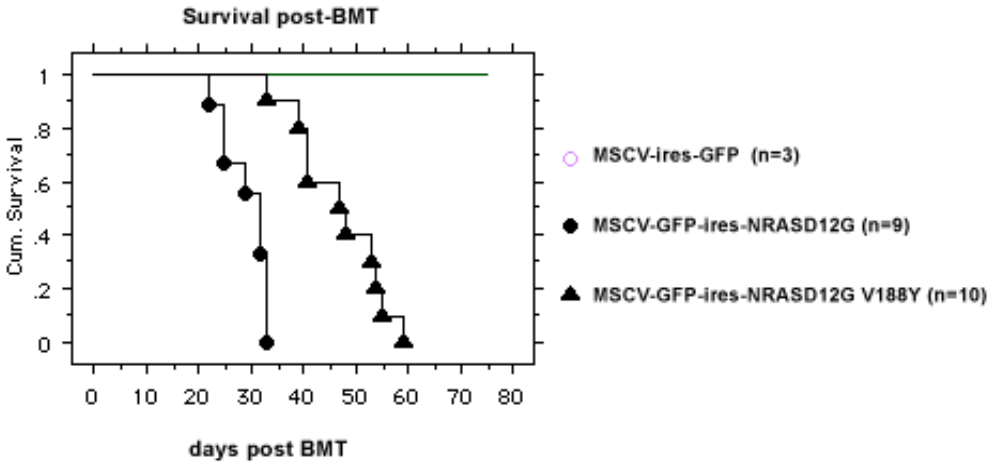

Figure 3. Survival of mice receiving transplantation of oncogenic NRAS (NRASD12), the A2 mutant of NRASD12, or GFP control retroviruses transduced BM cells from 5-FU treated BALB/c mice. Survival curves were generated by Kaplan-Meier survival analysis. The number (n) of mice for each group is indicated.

We have previously found that the Grb2 SH2 binding site Y177 of BCR-ABL, the major mediator of BCR-ABL inactivating RAS, is required for the induction of CML-like disease by BCR-ABL. Recently we further show that oncogenic RAS can rescue the defect of the Y177F mutant BCR-ABL in the induction of CML-like disease. We will test the importance of the AAX peptide cleavage and methylation of RAS in BCR-ABL leukemogenesis using the A2 mutant of oncogenic RAS.

Key research accomplishment

• We have generated $Rce1^{flx/flx}$ or $Icmt^{flx/flx}$ mice, as well as $Rce1^{flx/+}$, $Icmt^{flx/+}$, $Rce1^{+/+}$, or $Icmt^{+/+}$ control mice harboring the Mx1-Cre transgene.
• We have found that Icmt plays a critical role in BCR-ABL leukemogenesis. This finding suggests that Icmt may be an effective target for developing CML therapeutics.
• We have found that the role of Rce1 in BCR-ABL leukemogenesis is dosage dependent.
• We have found that the mutation affecting the AAX peptide cleavage and methylation of RAS *in cis* significantly reduces RAS leukemogenesis, suggesting that the AAX peptide cleavage and methylation play an important role for RAS leukemogenesis.

Reportable outcomes
Not yet.

Conclusion

Our results show that Icmt plays an important role in the pathogenesis of CML and suggest that this enzyme is an effective target for developing CML therapies. Our results also show that the role of Rce1 in BCR-ABL leukemogenesis is dosage dependent.

References
NA

Appendices
NA